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# Perinatal exposures to rotating magnetic fields 'demasculinize' neuronal density in the medial preoptic nucleus of male rats

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**Abstract**

Pregnant rats were exposed continuously for 3 days before to 3 days after birth to 0.5 rotating magnetic fields (RMF) whose intensities ranged between 1.5 and 3.0 mT or between 50 and 300 $\mu$T or to sham field conditions. When the male and female rats exposed to these perinatal conditions were about 100 days old, the numbers of neuronal soma and the numbers of nuclei for the three major types of glial cells were counted within the medial preoptic nucleus (MPO), suprachiasmatic nucleus (SCN) and ventromedial nucleus (VMH) of the hypothalamus. The male rats but not the female rats that had been exposed to either intensity of the RMF showed a significant reduction (similar to normal females) in the numbers of neurons within the MPO; the differences accommodated one-third of the variance and were not reduced significantly when the cell densities of the VMH or SCN were covaried before the analyses. The results suggest that some sexually dimorphic structures may be permanently and differentially affected when exposed perinatally to relatively weak extremely low frequency magnetic fields. © 1998 Elsevier Science Ireland Ltd. All rights reserved

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Sexual dimorphism within the mammalian brain is manifested within multiple structures [7]. The normal ontogenetic development of these nuclei is strongly dependent upon the availability of the appropriate sex hormones within the first few hours around birth [5,6,8,15]. Prenatal exposure to a low frequency magnetic field has been shown to demasculinize adult scent marking behavior in male rats [9]. This result suggested that perinatal exposures to extremely low frequency magnetic fields could permanently alter the density of neuronal soma within sexually dimorphic nuclei. The following experiment was designed to test this hypothesis.

We examined the numbers of neuronal soma and the numbers of nuclei of the primary types of glial cells within the medial preoptic nucleus (MPO), suprachiasmatic nucleus (SCN) and ventromedial nucleus (VMH) of the hypothalamus (as defined by Paxinos and Watson [10]) of male and female adult rats that had been exposed perinatally to an 0.5 Hz rotating magnetic field (RMF). These three structures were selected because they have been associated with the behaviors [2] displayed by rats exposed perinatally to the RMF. They include increased emotional reactivity [11] and facilitated acquisition of tasks associated with aversive stimuli [13,14].

A total of 15 pregnant, 90 to 130-day-old Wistar female albino rats (obtained from Charles River, Quebec) were exposed continuously from 3 days before parturition to 3 days after parturition (total of 7 days exposure) to either the RMF or sham field conditions. Although the details of the RMF device have been described elsewhere [14], it is essentially two horseshoe magnets that are rotated about their major axes in opposite directions by a pulley system driven by an electric motor. There were two exposure intensities that were determined by the distances from the RMF. These magnitudes ranged, within the plastic ($27 \times 27 \times 27$ cm) housing cages (covered by plastic mesh), between 1.5 and 3.0 mT and between 50 and 300 $\mu$T. The sham field exposure involved placing the females within the same positions after the magnets had been removed but while the equipment was operating.

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During the exposure period the light-dark cycle was 12:12 h with the local onset at 0800 h. The temperature was controlled to within 1°C of 21°C. Each female was housed in a plastic cage with food and water available ad libitum. On the fourth day postnatally, litters were culled to eight pups (four male and four female whenever possible). The litters were then removed from the experimental setting and returned to the holding areas where they were maintained in maternal housing. At weaning, 21 days of age, three rats of the same sex were housed three per cage.

When the rats were between 90 and 115 days old a male and female from each of the five litters exposed to the high intensity RMF, the five litters exposed to the low intensity RMF and five litters exposed to the sham field ($n = 5$) were randomly selected (total $n = 30$). After their brains were removed within 4 min following decapitation and fixed in ethanol-formalin-acetic acid [12], the cerebrums were processed and embedded in paraffin. Ten micrometer sections were obtained by microtome from three coronal sections that sampled along the longitudinal axis of the MPO and VMH and for two coronal sections of the SCN.

The sections were stained with toluidine blue O. This fixative and stain produced very clear details that allowed discrimination of the nuclei of the three types of glial cells and demonstrated acute details of neuronal soma [12]. Under oil immersion at 1000×, the numbers of neurons (defined as neurons with a least a portion of the nucleus present) and the numbers of nuclei for astroglia, oligodendroglia and microglia for two microscopic fields within the upper 1/3, middle 1/3 and lower 1/3 for each structure for the three slides were counted. The positions of the MPO, VMH and SCN were verified by Paxinos and Watson [10]. Because we were examining cell density and not the cross-sectional area of these nuclei, each field was counted three times to insure intrarater reliability and the median value was used as data. The morphological characteristics of the glia nuclei were sufficient for intrarater and interrater (with an experienced histopathologist) reliability coefficients of $rs > 0.90$.

Two-way analyses of variance (ANOVA) were completed for the mean numbers of neuronal soma and numbers of nuclei for the three types of glia cells as a function of exposure (RMF vs. control) and sex. To discern potential intensity dependence a second analysis was completed as a function of the three intensities (high, low and sham) and sex. To help verify the specificity of the statistically significant interaction between sex and perinatal treatment, analyses of covariance were first completed for each structure by removing the shared variance with the other two structures before the analyses of variance were completed. To verify the strength of the effect, a three-way analysis of variance (MANOVA) with one level repeated (numbers of neurons in the SCN, MPO and VMH) and two not repeated (sex, field strength) was completed. These and ancillary analyses involved SPSS software.

The means and standard deviations for the numbers of neuronal bodies and the numbers of different glial nuclei per $mm^2$ for males and female exposed perinatally to the RMF or to sham field conditions are shown in Table 1. The results of the ANOVAs indicated that only the numbers of neurons (but not the nuclei for any of the types of glial cells) within the MPO and VMH displayed statistically significant ($P < 0.05$) group differences. The significantly ($F(1,26) = 5.01$, $P < 0.05$; $\eta = 0.37$) fewer neurons within the MPO of rats exposed perinatally to the RMF was found to be due to the statistically significant interaction ($F(1,26) = 4.61$, $P < 0.01$) between sex and the type of perinatal exposure. Post-hoc analyses (Tukey, $P < 0.05$)

Table 1

Mean ± SD for the numbers of neurons and numbers of glial cell nuclei per $mm^2$ within the medial preoptic (MPO), suprachiasmatic nucleus (SCN), ventromedial nucleus (VMH) or the caudal shell of the VMH for male and female rats exposed perinatally to the RMF or to sham field conditions

| Structure | Sham field-exposed | | RMF-exposed | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| MPO | | | | |
| Neurons | 521 ± 41[a] | 407 ± 78[b] | 413 ± 80[b] | 410 ± 44[b] |
| Oligodendrocytes | 57 ± 16 | 58 ± 21 | 55 ± 16 | 61 ± 10 |
| Astroglia | 95 ± 8 | 85 ± 28 | 81 ± 18 | 92 ± 18 |
| Microglia | 4 ± 5 | 5 ± 3 | 8 ± 7 | 4 ± 7 |
| SC | | | | |
| Neurons | 799 ± 165 | 756 ± 131 | 793 ± 100 | 778 ± 86 |
| Oligodendrocytes | 56 ± 18 | 50 ± 19 | 50 ± 20 | 54 ± 21 |
| Astroglia | 73 ± 8 | 71 ± 11 | 75 ± 17 | 81 ± 21 |
| Microglia | 8 ± 13 | 11 ± 10 | 8 ± 11 | 4 ± 10 |
| VMH | | | | |
| Neurons | 445 ± 61[a] | 461 ± 89[a] | 403 ± 30[b] | 416 ± 32[b] |
| Oligodendrocytes | 62 ± 18 | 96 ± 33 | 78 ± 28 | 79 ± 18 |
| Astroglia | 122 ± 18 | 132 ± 20 | 114 ± 25 | 111 ± 17 |
| Microglia | 1 ± 1 | 9 ± 9 | 9 ± 9 | 5 ± 5 |

a versus b, $P < 0.05$.

indicated that the source of the strength of the interaction explained 31% of the variance in numbers of neuronal soma ($\eta = 0.56$, $P < 0.05$) and was due to the absence of statistically significant differences between the treatments for the females but significantly fewer numbers of neurons within the MPO for the males that had been exposed to the RMF (regardless of intensity) relative to sham field conditions.

The males and females exposed to the RMF perinatally also displayed fewer numbers of neurons within the VMH ($F(1,26) = 4.88$, $P < 0.05$; $\eta = 0.40$). There was neither a significant sex difference ($F < 1$) nor an interaction between sex and perinatal treatment ($F < 1$). If the shared variance in numbers of neuronal soma within the VMH and SCN was first removed from the MPO (analysis covariance), the interaction between sex and perinatal magnetic field exposure for the numbers of neurons within the MPO was still statistically significant ($F(2,22) = 6.14$, $P < 0.01$). Covariance for the other two structures before ANOVA did not change the absence of a statistically significant interaction between field treatment and sex for the VMH ($F(2,22) = F < 1.00$) or SCN ($F < 1.00$). The repeated measure ANOVA verified the statistically significant interaction ($F(4,48) = 2.24$, $P < 0.05$; $\eta^2 = 16\%$) between the neuronal density within each of the three structures, gender and the three levels of field intensity (sham, low-RMF, high-RMF).

To examine possible 'intensity-dependence' of the interaction between sex and field exposure, a second analysis was completed as a function of gender and the exposures to the 1.5 to 3.0 mT, 50 to 300 $\mu$T or sham field conditions. Post hoc examinations of the statistically significant one-way ANOVA ($F(5,24) = 3.30$, $P < 0.05$) indicated that the males exposed to either of the two RMF intensities displayed fewer cells in the MPO relative to the normal males; this discrepancy was not significant for the MPOs of females. The means and standard deviations for the numbers of neurons within the MPO for males and females exposed to either the high intensity, low intensity or sham-field conditions are shown in Fig. 1.

The results of this study are consistent with the hypothesis that perinatal exposures to extremely low frequency magnetic fields can differentially affect the density (numbers) of neurons but not glial cells within sexually dimorphic nuclei such as the medial preoptic nucleus (MPO) of the hypothalamus. The absence of statistically significant interactions between sex and perinatal magnetic field exposure for the density of neurons within the SCN or VMH and the maintenance of this interaction for the MPO only following covariance (even after the shared variance for both VMH and SCN was first removed), strongly indicate the MPO effect was not due to non-specific, confounding measures of cell density. This conclusion is strengthened by the fact that the statistically significant differences were found only for the density of neuronal soma and not for the density of any of the three types of glial nuclei and were evident for the neuronal numbers even when all three structures were analyzed simultaneously.

The comparable numbers of neuronal soma within the MPO of females exposed perinatally to the magnetic fields or to the sham-field condition compared to the males exposed perinatally to the magnetic fields also suggest that the effects were not an artifact of experimental procedures but specific to the exposed males. Both the direction and the strength of the interaction between sex and perinatal exposure to the RMF for the numbers of neurons in the MPO were compatible with the differential effect of these exposures upon the open field behaviors of male but not female rats [11] and were similar in magnitude to the effects of prenatal stress upon the play patterns of male but not female rats [4].

The similarity of the numbers of neurons within the MPO for males exposed to either the low intensity or high intensity RMF, both of which differed significantly from normal (sham-field) males, also indicates that the threshold intensity to evoke this effect may be below 50 $\mu$T. Although extrapolation from rodent to human development must be pursued cautiously, Hodge and Persinger [3] found that the weak but statistically significant canonical correlation between a cluster of temporal lobe (limbic) indicators and geomagnetic activity during the time of parturition was evident only for university men but not for university women. Because the functional developmental stage at birth differs for humans and rats the effects of perinatal magnetic stimuli would be expected to influence a process that may contribute to similar changes in synaptic organization [5] within the developing limbic system.

The direction of the experimental effect for the present study can be interpreted as demasculinizing or 'feminizing' because the neuronal density for the male rats exposed to the RMF did not differ significantly from the values for normal female rats. Immobilization of pregnant females and nutritional stress during gestation have also been shown to

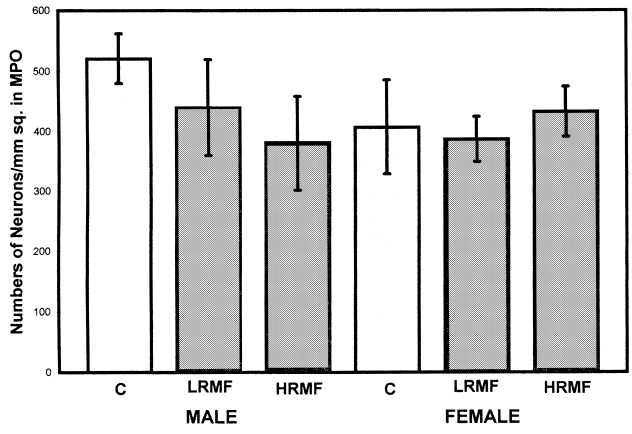


Fig. 1. Numbers of neurons/mm$^2$ within the medial preoptic nucleus of the hypothalamus for adult male and female rats who were exposed perinatally to various intensity 0.5 Hz, rotating magnetic fields. LRMF (50–300 $\mu$T); HRMF (1.5–3.0 mT); C (sham field-exposed). Vertical bars represent SDs (divide by 2.2 for SEMs).

decrease the area of the preoptic region in male but not female rats [1]. The Hodge and Persinger study [3] involving human subjects also showed a 'geomagnetic intensity-dependent' enhancement of androgyny or greater feminization of the subjective experiences for only the men.

Perinatal exposure to the RMF was also associated with a general effect that was not differential for sex. Both male and female rats that had been RMF-exposed perinatally displayed 10% fewer neurons within the VMH compared to non-exposed rats. Although the induction of different types of effects by perinatal magnetic field exposure within different cells systems would be expected in a complex matrix such as the developing brain, these empirical results again emphasize the importance of realizing the presence of multiple, parallel effects within developing cell systems exposed to time-varying magnetic fields with even simple temporal shapes.

Although we did not complete a cross-sectional study (which would require measurements from brains of different ages), we reasoned that the quantitative reduction in neurons within the VMH for both male and female rats and within the MPO for the male rats that had been exposed perinatally to the RMF was not ontogenically recent. If these changes had been recent we would have expected significant differences between groups for the numbers of glial nuclei because of the commencement of either reactive gliosis or phagocytic activity. The absence of hamartomas (increased clusters of astroglial) is also consistent with a selective failure of neurons to survive within the male MPO or within the male and female VMH rather than the occurrence of focal microlesions or abrupt trauma.

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